Exhibit 5

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Warfarin treatment of chronic idiopathic urticaria and angio-oedema

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Summary

Background Chronic idiopathic urticaria is a disabling condition that does not always respond to antihistamine drugs and other agents are sometimes needed to control disease activity. Warfarin has demonstrated efficacy in single unblinded case studies [1] but has been dismissed by others [2].

Objective We investigated the effect of warfarin treatment in eight patients with chronic idiopathic urticaria unresponsive to antihistamines in an open study. Six of the eight patients responded to treatment and three had a dramatic response. These three were included in a double-blind placebo-controlled trial of warfarin therapy to confirm significant benefit from treatment.

Methods The three warfarin responders had their stable warfarin dose encapsulated and placebo capsules were provided. A double-blind placebo-controlled crossover trial was performed on each patient. Visual analogue scores recorded disease activity.

Results Comparison of visual analogue scores showed a significant benefit while on warfarin with a reduction in pruritus and angio-oedema.

Conclusion This is the first double-blind placebo-controlled study to show a response of chronic idiopathic urticaria to warfarin. The mechanisms of action are unclear and require further study.

Keywords: treatment, urticaria, warfarin

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Introduction

Chronic idiopathic urticaria is a common disorder characterized by recurrent urticarial weals of unknown origin for 3 or more month's duration. Typically there is a variable response to antihistamines and a tendency to spontaneous resolution; many patients are adequately treated by general practitioners. However there is a subgroup of patients in whom there is no tendency to improvement with time and who suffers severe urticaria often accompanied by angio-oedema, which is unresponsive to antihistamines. Some of these patients have an associated underlying disorder but most have no detectable abnormality. Severe urticaria is a debilitating condition and nonantihistamine treatments are limited by their lack of efficacy and/or risk of side effects. It has been suggested that some patients may respond to warfarin therapy [1] but this was questioned in a further study which showed no improvement [2]. The possibility that in some forms of urticaria proteases of the complement, kinin, and clotting or fibrinolytic systems are activated to generate vasoactive mediators encouraged us to examine the effects of warfarin in chronic idiopathic urticaria.

We first performed an open study of eight patients with treatment resistant urticaria who appeared to show significant clinical benefit. We then performed a double-blind placebo-controlled cross-over study on three of these patients and confirmed a major therapeutic response. To explore the underlying mechanism, patients were challenged with mast cell degranulating agents: compound 48/80 and histamine both on and off warfarin.

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Materials and methods

Open study

Initially eight patients with a clinical diagnosis of angioedema and chronic idiopathic urticaria without physical precipitating factors or systemic features were studied because their disease was resistant to full dosage of antihistamines. Urticarial vasculitis was not formally excluded by biopsy. All drugs including antihistamines were stopped 1 week prior to the trial. They were asked to assess their global symptoms on a daily basis using a 20-cm linear visual analogue scale where 0 = no symptoms and 20 = worst symptoms ever experienced. Global scores represented general disease activity and well-being. Mean scores were obtained by measuring the actual distance from the origin to the point marked on the scale by the patient. A new scale was used each day and the patients brought all the scales with them for weekly review. The pretreatment assessment period lasted for 3 months after which each patient was anticoagulated with warfarin (there were no clinical contraindications) to achieve an International normalized ratio (INR) between 2.0 and 2.5. Patients were assessed for a further 3 months on treatment and the relative visual analogue scores compared.

After a washout period where the INR returned to normal there was a further assessment of 2 months without treatment. Visual analogue scores were analysed with the Wilcoxon rank sum test as the data derived from the visual analogue scores was not an interval or ratio scale.

Effect of warfarin on response to histamine and compound 48/80

Histamine in doses of 15.6, 62.5, 250, 1000, 4000, and 16000 ng and saline control was injected intradermally into the volar forearm of patients. Weal diameter, skin fold thickness, determined by Harpenden callipers, and erythema, measured with a reflectance meter (Diastron) were compared whilst the patients were fully anticoagulated (INR 2.0–2.5) with warfarin and off warfarin with a normal INR. All patients were off antihistamine treatment for one week prior to challenge. Similarly the same subjects were challenged on and off warfarin with 5, 50, 500, 5000, and 50 000 ng of compound 48/80. Significance was determined with Students paired *t*-test.

Double-blind placebo-controlled study

Three patients from the open study showed dramatic clinical improvement in their symptoms. To confirm that this was a real effect due to therapy, a double-blind placebo-controlled trial was performed. The three patients described below had been shown to have a stable INR on their individual warfarin doses. In conjunction with the pharmacy department at the Royal Liverpool Hospital each patient had their daily individual warfarin dose put into one gelatine capsule. A placebo capsule, identical in appearance, was also provided and the patient took either according to a protocol held by the pharmacy. The trial was conducted in a double-blind placebo-controlled fashion with the pharmacy acting as the third party unblinded dispenser. Patients were randomly allocated to a series of four bottles of capsules – two active and two placebo which they could encounter in any order. There were enough capsules in each bottle for one month's supply taking one capsule a day.

Over the ensuing four months patients were asked to complete weekly 20 cm visual analogue scales to assess their angio-oedema and pruritus. Urticarial lesion number was not assessed. A weeks washout period was given after each change of treatment to allow for levels of warfarin to reach the therapeutic range or to allow levels to fall back to the normal range before scores were taken. Weekly blood for INR measurements were taken irrespective of treatment and were sent to an independent observer blinded to the protocol and treatment to ensure anticoagulation remained within safe limits. Patients were also examined weekly for clinical signs of disease activity and complications of warfarin treatment, but global scores by the examining physician were not made. At the end of the trial the code was broken and responses were compared with treatment groups. The data from the visual analogue scores were analysed statistically by use of the Wilcoxon rank sum test.

In an attempt to characterize this group of patients we performed additional tests. Whilst off all treatment for at least 1 week all patients were inoculated with autologous pretreatment serum as previously described to look for the presence of serum-derived mast cell degranulating factors [3].

Patients

Mr TW a previously fit 38-year-old man presented with a 3-year history of almost daily recurrent facial swelling especially around the eyes and frequent severe urticarial swelling on the body. Individual lesions would last for up to 24 h and fade without trace. There was no family history of urticaria or angio-oedema. He had tried many antihistamines unsuccessfully in standard and high dose and for the previous 2 months a combination of Cimetidine 400 mg b.d. and Loratidine 10 mg o.d. with no benefit. The urticaria was unrelated to any physical factors and all investigations including C1 esterase inhibitor levels and complement levels were normal. Initially he was admitted to hospital for an unsuccessful trial of a strict exclusion diet. Subsequently he was started on warfarin as described and he improved dramatically. Through trial and error it was found

that when his INR (normal = 1.0) fell below 2.0 his urticaria would flare but above this level he would remain virtually symptom free. He has now been controlled on 6 mg warfarin with an INR between 2 and 2.5 for the last 2 years with severe flares of his facial swelling/urticaria should his INR fall substantially. He has suffered no warfarin-related side effects. He agreed to take part in the double-blind placebo-controlled trial.

Mrs KD a 38-year-old lady had suffered from angio-oedema and urticaria for 5 years and during this time she was never completely free of lesions. Urticarial lesions occurred on any area of the body, unrelated to physical stimuli, would last for about 12 h and disappear. She was not helped at all by standard dose and even high dose (Loratidine 30 mg/day) antihistamines and she was otherwise well on no drugs. Routine blood tests, complement and C1 esterase inhibitor levels were normal. She was started on warfarin as a therapeutic challenge. Her symptoms quickly diminished so that by week 2 she was completely free of any lesions. She was stabilized on a steady warfarin dose and she agreed to take part in the double-blind placebo-controlled trial.

Mrs PQ a 54-year-old woman presented with a 3-year history of almost constant crops of urticarial weals on the body and recurrent pruritic facial swelling involving her eyes and mouth. Standard dose antihistamines coupled with cimetidine 800 mg per day limited the attacks to 3–4 per week but she still felt that this was intolerable. She was otherwise well, on no drugs and there was no obvious relationship with physical stimuli.

She was commenced on warfarin and her antihistamines were stopped. As her INR increased she slowly improved and she found that if her INR rose above 3.0 she was completely symptom free. However she developed a sub-conjunctival haemorrhage when her INR was 3.7 and whilst she subsequently had an INR between 2.0 and 2.5 her symptoms were reduced but tolerable. This necessitated warfarin 2 mg per day and at this point she agreed to enter the double-blind placebo-controlled trial.

Results

Open study

Six of the eight patients showed a good clinical response whilst on warfarin, two showed no clinical response. Overall using results from all eight patients in the open trial the benefit derived from warfarin was significant – mean visual analogue score of global symptoms before treatment 14.5 (SD 6.5); mean visual analogue score on treatment: 4.5 (SD 7.9). ($P = 0.017$ 96.1% CI −16 −3 Wilcoxon rank sum test).

There was no significant difference in cutaneous skin fold thickness, weal diameter or erythema measured by the reflectance meter to either histamine or compound 48/80 on or off warfarin. For example when comparing weal diameter 10 min after cutaneous challenge with histamine on and off warfarin results were not significantly different: $P = 0.06$ (Students paired t-test) see Fig. 1. After challenge with compound 48/80 there was no significant difference in weal diameter at 10 min: $P = 0.3$ (Students paired t-test) see Fig. 2.

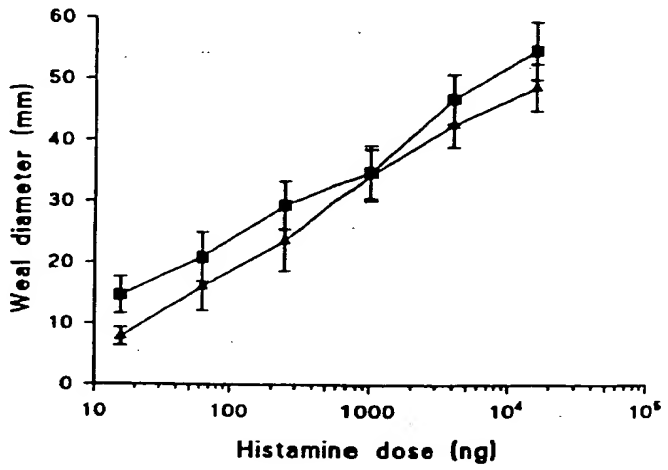

Fig. 1. Effect of warfarin on histamine weal diameter ($n = 8$). Off warfarin (■); on warfarin (▲).

Double-blind placebo-controlled study

Comparison of visual analogue results for angio-oedema on active and placebo treatment showed a significant benefit

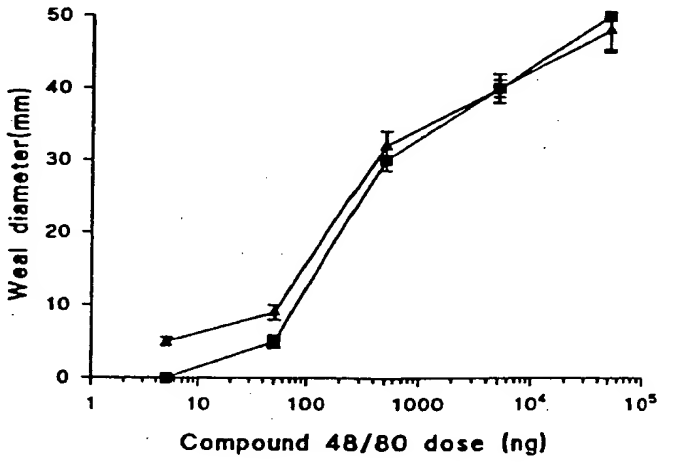

Fig. 2. Effect of warfarin on compound 48/80 weal diameter ($n = 8$). Off warfarin (■); on warfarin (▲).

while on warfarin: mean score on placebo, i.e. the two periods off active treatment was 17.67 whereas mean score on warfarin, i.e. the two periods on active treatment was 5.02 ($P = 0.031$ 96.9% CI−18−5.8 Wilcoxon rank sum test). Similarly, pruritus was greatly reduced: mean values of 16.97 for placebo and 5.05 for active treatment. ($P = 0.031$ 96.9% CI−21.6−6.7 Wilcoxon rank sum test). This confirmed a statistically significant benefit of warfarin treatment both for the angio-oedema and pruritus aspects of the condition.

In all three patients injection of autologous serum gave responses indistinguishable from saline control.

Discussion

The possibility that some patients with chronic idiopathic urticaria would derive clinical benefit from warfarin was originally suggested by Ryan [4] and supported clinically with anecdotal evidence [1,5]. Evidence supporting this was obtained in our open study in which six of eight patients with antihistamine resistant chronic idiopathic urticaria showed clinical benefit. However since chronic idiopathic urticaria is often a variable condition, showing periods of reduced activity or even spontaneous remission we felt it necessary to confirm this was a real therapeutic effect. Therefore a randomized placebo-controlled double-blind trial was performed on three of the patients who showed the most complete resolution of symptoms during the open study. This suggested that in these patients there was a significant beneficial effect of warfarin treatment. As shown with the formal challenge with histamine and compound 48/80, the effects of warfarin were not due to modification of responses to histamine and other mast cell mediators responsible for the acute weal and flare.

The known actions of warfarin are to reduce protein C concentrations and inhibit synthesis of vitamin K-dependent proteins in the clotting cascade (prothrombin and factors VII, IX and X). Warfarin acts as a competitive inhibitor of vitamin K and during the carboxylation of the precursors of these factors, vitamin K is converted to its inactive oxide and then metabolized back to its active form. Warfarin prevents this reconversion. The possibility that activation of clotting or fibrinolytic pathways as a mechanism in angioedema or urticaria was suggested by Ryan. He postulated that plasmin may contribute to the development of urticaria by removing the 'fibrin film wall', by activating complement and by increasing production of fibrin degradation products. However Smith *et al.* provided evidence against the involvement of plasmin [6].

The protein C/S anticoagulant pathway has been proposed to be a common link between coagulation and inflammation and an endothelial cell protein C receptor, modulated by inflammatory cytokines may play a part in this [7]. Activated protein C up-regulates interleukins 6 and 8 and may block neutrophil activation [8]. Warfarin inhibition of thrombin production also contributes to the anti-inflammatory action as in addition to short-term endothelial activation via P-selectin and platelet activating factor release stimulating early neutrophil adhesion and activation, thrombin induces E-selectin and interleukin 8 secretion in human vascular endothelium, facilitating a long acting pro-inflammatory response with neutrophil activation and extravasation [9]. There is convincing evidence that adhesion molecule expression is an important early event in chronic idiopathic urticaria and delayed pressure urticaria facilitating neutrophil infiltration of tissue [10]. Downregulation of these molecules by warfarin may impair vascular endothelial activation and lead to clinical improvement. It has been suggested that differential endothelial adhesion molecule expression may contribute to the pathogenesis of fleeting vs persistent weals [11] and it is of interest that in our patients the clinical impression was of a tendancy for benefit to be maximal against persistent angio-oedematous lesions rather than fleeting weals. This may indicate that warfarin preferentially downregulates certain adhesion molecules important in sustained urticarial/angio-oedema reactions.

Another possibility is that warfarin may modify effects of the proteases in the complement or kinin generating cascades. These processes are important in C1 esterase inhibitor deficiency when activation of C1 generates small vasoactive peptides resulting in vasodilatation and oedema. Also immune/allergic reactions can activate the kallikrein-mediated generation of kinins. One inhibitor of kinin production has been tried successfully in chronic urticaria (Trasylol) [10]. Trasylol inhibits certain proteolytic enzymes including kallikrein—an important kinin-derived from circulating prekallikrein. In high doses Trasylol suppresses C1 esterase and inactivates kallikrein precursors. This is helpful in hereditary C1 esterase inhibitor deficiency which gives rise to angioedema. Pre-kallikrein is activated by a variety of factors including factor XIIa and plasmin. One can easily hypothesize therefore that warfarin may inhibit plasmin activity thereby reducing activation of kallikrein and lowering the tendency to increased vessel permeability, tissue oedema and thus, urticaria. However, there is no evidence of raised kinin levels in urticaria or of low levels of endogenous inhibitors so the mechanism of action remains obscure. There is no doubt however, that our three patients derived and continue to have considerable benefit from the drug. They may represent a small subset of patients with chronic urticaria who respond favourably to this treatment though the mechanism is unknown. However we could not identify any features in these patients that would allow prediction of a good response to warfarin. In particular we could not detect the presence of mast cell degranulating factors in autologous serum in these patients, so this subset appears

not to have the anti-IgE receptor antibody. Further studies are required with larger numbers to determine the characteristics of those patients who do respond. Effects of warfarin on mast cell derived proteases, and the activation of platelets and leucocytes are potential targets. This may provide clues to the mechanism of the weal induction in these patients. If warfarin were to be used in the treatment of angio-oedema/urticaria, then its use should be limited to cases were conventional therapy has failed as the incidence of major haemorrhage is approximately 7% [12], although risk can obviously be minimized by avoiding anticoagulation in high risk cases such as alcohol abuse, chronic renal insufficiency and previous gastro-intestinal haemorrhage.

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